

FIGURE 1

APGK12 (SEQ ID NO 1) Replicon RNA compared to I377/NS2-3' Replicon RNA

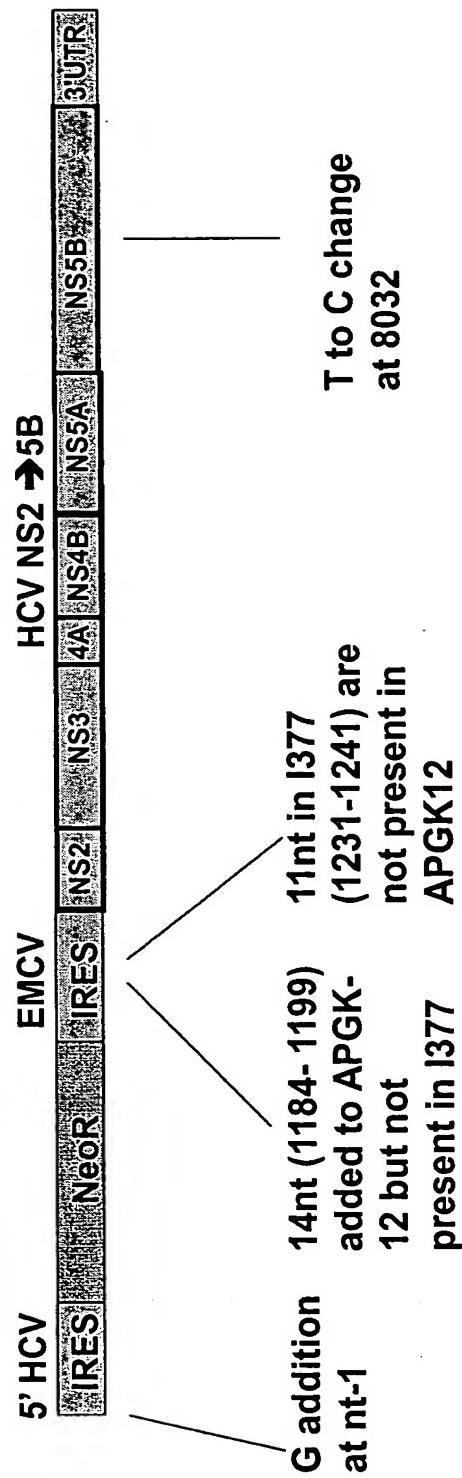
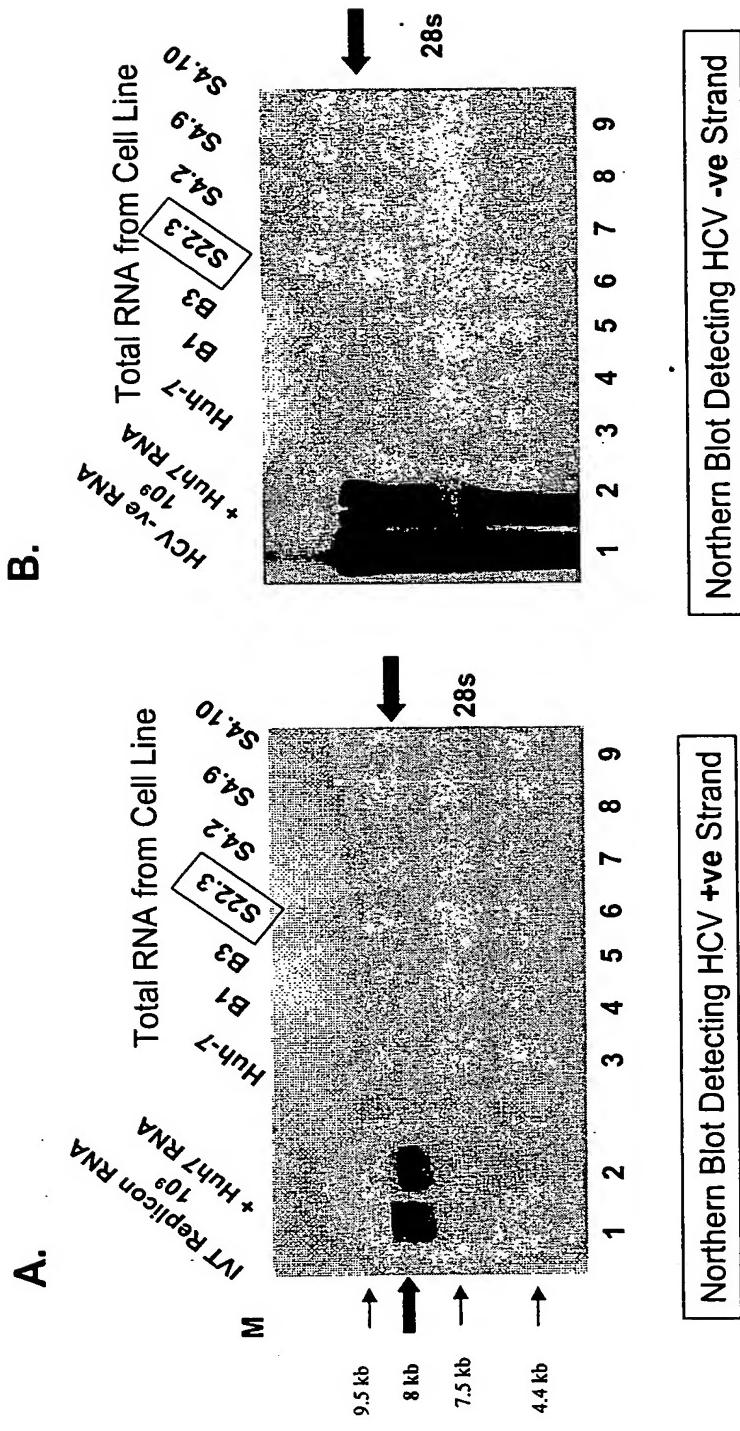


FIGURE 2



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FIGURE 3

A.

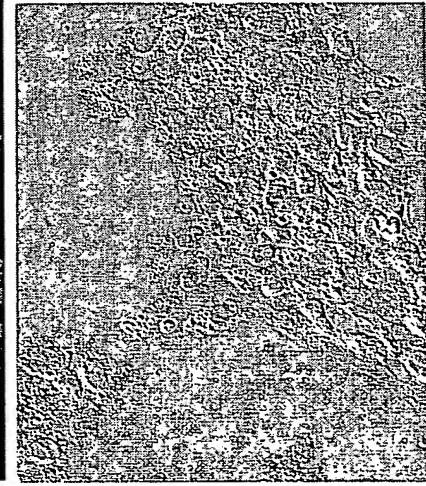
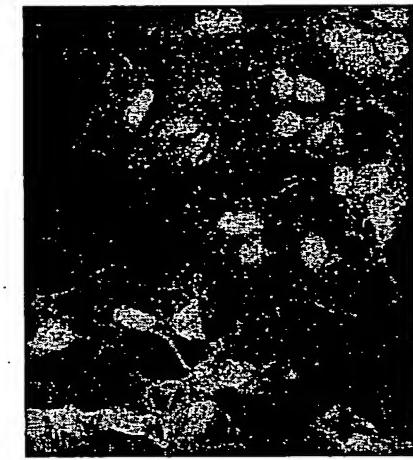
S22.3



B.

B1

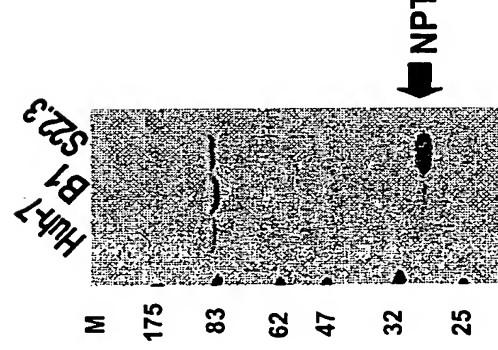
Anti-HCV NS4A
Immunofluorescence



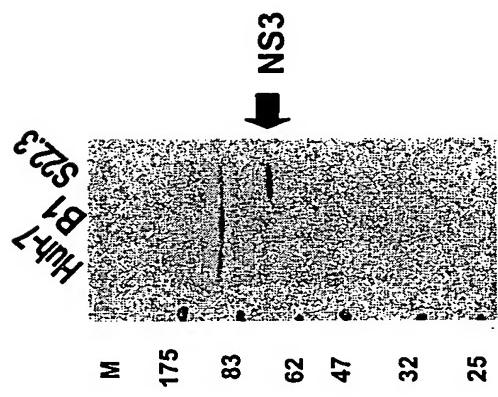
DIC Microscopy

FIGURE 4

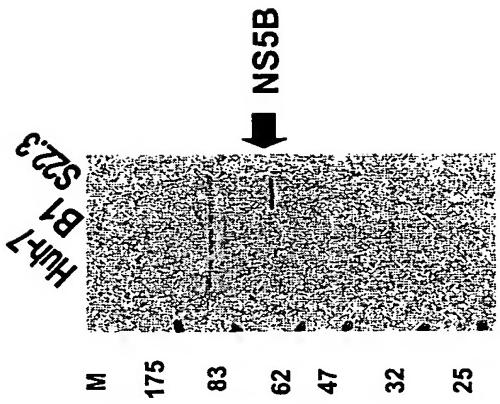
A.



B.



C.



Anti- Neomycin
Phosphotransferase

Anti- HCV NS3

Anti- HCV NSSB

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FIGURE 5A

S 22-3 SEQ ID NO.2	R3 SEQ ID NO.4	R3-rep SEQ ID NO.7	R7 SEQ ID NO.5	R16 SEQ ID. NO.6
5'end - FIRST nt (HCV IRES)	G (nt 1) A	G (nt 1) A	-	G (nt 1) A
NS 2	A (nt 161) G			
EMCV IRES	A (nt 1739) G			
NS 3				
	G (nt 2778) A	T (nt 2935) C	A (nt 2816) G	
	A (nt 2840) C	G (nt 2778) A	A (nt 2979) G	
	A (nt 4052) G	A (nt 2840) C		
		T (nt 3574) C		
		A (nt 4052) G		
NS 4A				
	A (nt 446) T	C (nt 446) G	C (nt 387) T	C (nt 475) T
	A (nt 446) T	C (nt 446) G	C (nt 446) G	C (nt 450) T
NS 4B		T (nt 4855) C	T (nt 4855) C	
NS 5A	G (nt 599) T	G (nt 535) G	A (nt 534) G	G (nt 549) C
	A (nt 6268) R	G (nt 549) T	G (nt 549) T	G (nt 632) C
		G (nt 5859) A	G (nt 5859) A	G (nt 6558) C
		C (nt 5841) T	C (nt 5833) C	C (nt 6601) C
		A (nt 6266) G	A (nt 6266) G	A (nt 6601) C
		T (nt 6115) G	T (nt 6115) G	T (nt 6601) C
NS 5B		A (nt 6652) G	-	C (nt 7252) T
				T (nt 8349) C

First nt = G from HCV virus

FIGURE 5B

	S 22-3 SEQ ID NO. 2	R3 SEQ ID NO. 4	R3 Rep SEQ ID NO. 7	R7 SEQ ID NO. 5	R16 SEQ ID NO. 6
5'end - FIRST nt (HCV IRES)	G (nt 1) A	G (nt 1) A	-	-	G (nt 1) A
NS 2	-	-	-	-	-
NS 3	-	R (1135) K S (1560) G	R (1135) K S (1560) G	E (1202) G	S (1148) G E (1202) G
NS 4A	K (1691) mix K/R	K (1691) R	K (1691) R	-	L (1701) F
NS 4B	-	-	-	-	-
NS 5A	T (1993) A G (2042) C	T (1993) A G (2042) C	T (1993) A G (2042) C L (2155) P	T (1984) V G (2042) R S (2404) P	G (2042) R
NS 5B	-	-	P (2166) L	P (2166) L	-
3'end : last 98 nt	-	-	-	M (2992) T	-

first a.a. of NS2 = 810

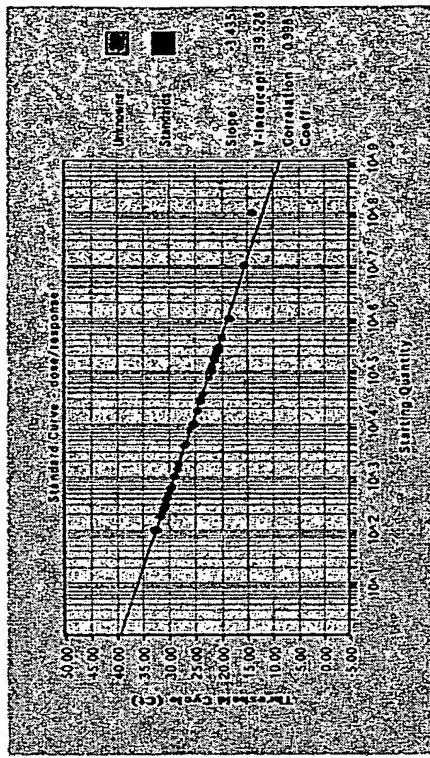
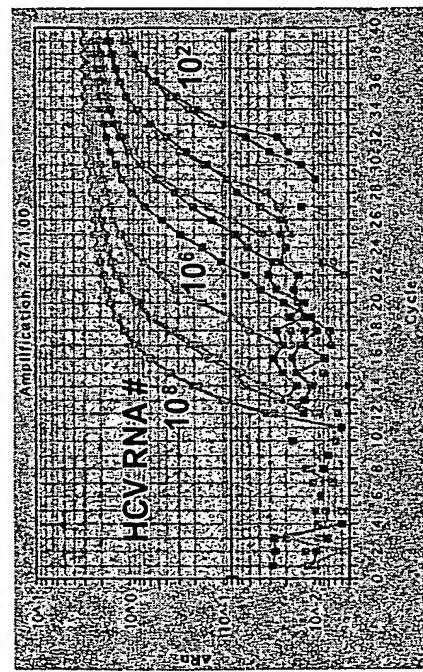
FIGURE 6
AMINO ACID SUBSTITUTIONS

CLONE APGK-12		AMINO ACID SUBSTITUTIONS											
5' HCV	EMCV	HCV NS2 → 5B		3' HCV									
IRES	NeoR	IRES	NS2	NS3	NS4B	NS5A	NS5B	UTR					
G (nt1) SEQ ID NO 1		-	-	-	-	-	-	-					
A (nt1) SEQ ID NO 24		-	-	-	-	-	-	-					
R3 rep A(nt1) SEQ ID NO 25		R(1135)K S(1560)G	K(1691)R	-	T(1993)A G(2042)C L(2155)P P(2166)L								
G(nt1) SEQ ID NO 7		R(1135)K S(1560)G	K(1691)R	-	T(1993)A G(2042)C L(2155)P P(2166)L								
77 cfu/ug		86 cfu/ug		1100000cfu/ug		2000000cfu/ug							

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FIGURE 7

HCV-Replicon: RNA Quantification



C_t = Threshold cycle α Starting RNA Quantity

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FIGURE 8

